

Using Groups of Regions

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Using Groups of Regions

Most samples, including two dimensional TLC plates, two dimensional electrophoresis gels, and tissue sections, can be quantitated by placing regions of interest (ROI) on the image.

You can quantitate ROIs by drawing groups of regions on the image. You can draw regions using a rectangle, ellipse, or an irregular shape. These tools are made available in the tool box of the **InstantImager** window when a region is selected.

You can organize the regions into groups. Each group is identified as T1, T2, etc. Each region within a group is identified with a tag. The region tag can be a number, the counts within the region, or a user-specified label. You can specify the tag type and the position of each tag relative to each region.

The **Edit Group of Regions** window (Figure 6-1) enables you to specify the parameters that determine how regions are tagged on the image and how results are reported.

Once the parameters have been defined at this window, you can create regions using the drawing tools.

To display the **Edit Group of Regions** window:

- ☐ Select **Create Template** from the **Analysis** menu.

Or,

- ☐ Select **EDIT** from the template bar.

The **Create Template** window is displayed.

- ☐ Select "Group of Regions" at the **Create Template** window.
- ☐ Select **OK**.

The selections at the **Edit Group of Regions** window are described below.

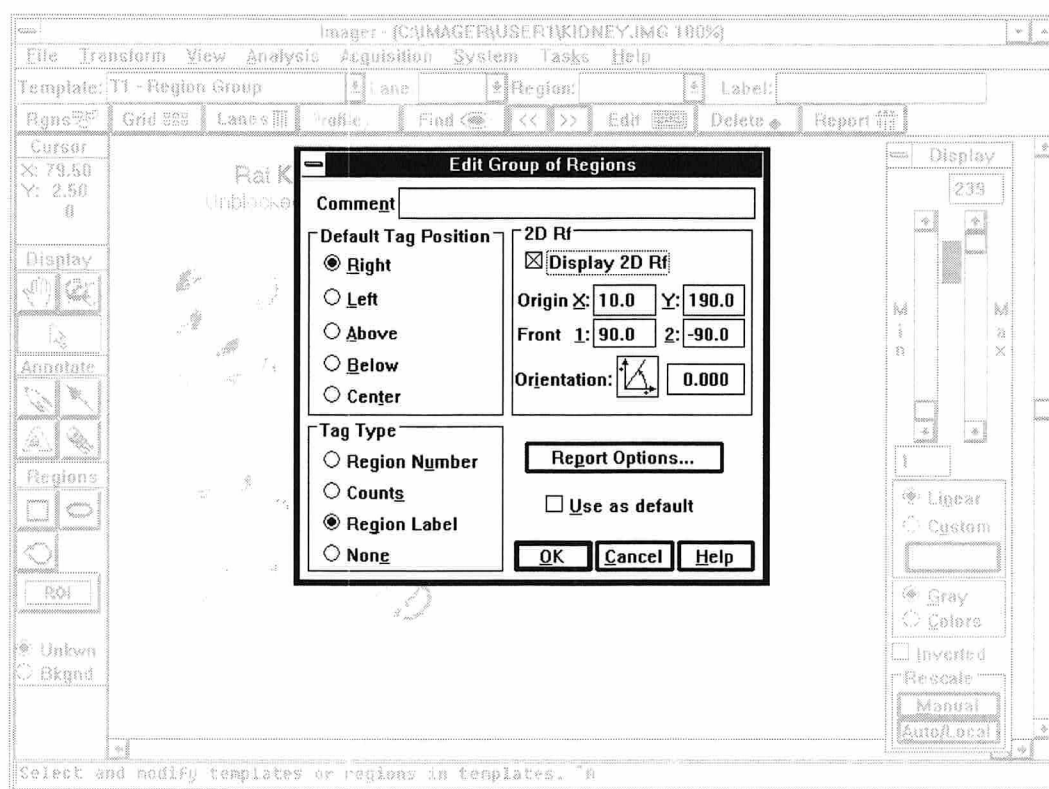


Figure 6-1. Edit Group of Regions Window.

Select **OK** after you have responded to the selections described below. Once you do, a template tag ("T1" for example) is displayed over the image. This tag represents the first region group. Use the drawing tools to create regions within this group (see *Region Drawing Tools*, page R224).

To create a second group of regions, revisit the **Edit Group of Regions** window and select **OK**. The next available template tag ("T2") is displayed over the image. This tag represents the second region group. You can draw regions within the second group.

An example region group is shown in Figure 6-2 .

<u>SELECTION</u>	<u>DESCRIPTION OF USE</u>
Comment	Enter a comment that will be included in reports.
Default Tag Position: Right, Left, Above, Below, Center	Select the placement of the tag relative to the each region: to the Right, Left, Above, Below, or in the Center.
Tag Type: Region Number	Select this option to have the system automatically number the regions in the group. The regions are numbered consecutively in the order they are created.
Tag Type: Counts	Select this option to have the system automatically display the gross counts next to each region.
Tag Type: Region Label	Select this option to display user-defined labels next to the regions.
Tag Type: None	Select this option if you want no region tags displayed or printed.

SELECTION**DESCRIPTION OF USE****Display 2D Rf**

Enables you to perform 2D Rf analysis. Once selected, all the other items in the 2D Rf section of the window will become active (grayed out otherwise). These options allow you to define the location and orientation of the two origins and fronts.

2D Rf: Origin X

Enter the X location on the image of the two origins. The X location is limited to values 0–240. You can edit the X location numerically or graphically.

2D Rf: Origin Y

Enter the Y location on the image of the two origins. The Y location is limited to values 0–240. You can edit the Y location numerically or graphically.

2D Rf: Front 1

Enter the distance of the first front from the location of the two origins. Front 1 is limited to values of +240 to –240. You can edit the distance of the first front numerically or graphically.

2D Rf: Front 2

Enter the distance of the second front from the location of the two origins. Front 2 is limited to values of +240 to –240. You can edit the distance of the second front numerically or graphically.

2D Rf: Orientation

Enables you to adjust the orientation of the 2D Rf region if the sample is not placed in the instrument with a precise horizontal–vertical orientation. The orientation is limited to values -360° to $+360^{\circ}$. You can edit the orientation of the 2D Rf region numerically or graphically.

SELECTION

DESCRIPTION OF USE

REPORT OPTIONS

Displays the **Region Group Report Options** window. Enables you to report the results from the regions. See the Chapter *Reporting Results* (page GS101) for more information on reporting results from regions.

Use as Default

Select the check box if you want the current parameters to be used every time you create a group of regions.

OK

Saves the parameters and closes the window.

CANCEL

Aborts the operation. Closes the window.

HELP

Displays online documentation that describes the function of the current window.

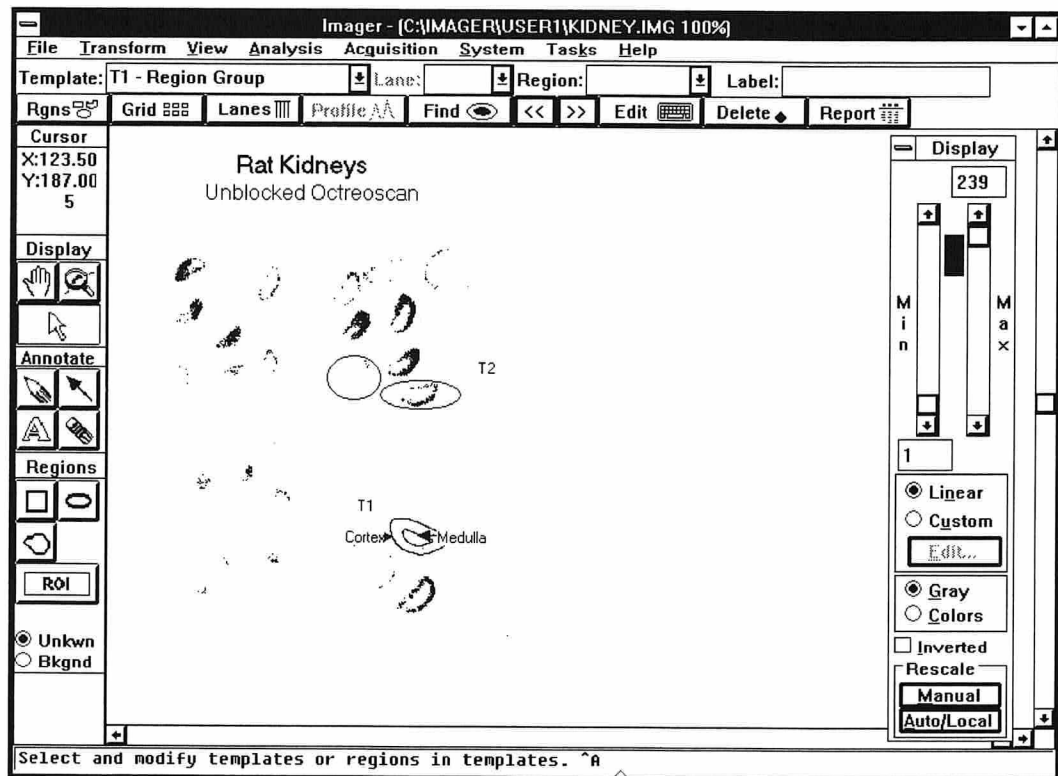


Figure 6-2. Region Group.

Region Drawing Tools

The region drawing tools will be displayed in the tool box when the group of regions is the selected template. The tools include:



The Rectangle tool enables you to draw rectangular shaped regions.



The Ellipse tool enables you to draw elliptical regions.



The Irregular tool enables you to enclose an ROI with an arbitrary shape.



The ROI tool enables you to draw a Region of Interest. A ROI defines the area in which the Find process will automatically create regions.

Single clicking and double clicking on a tool have different effects:

- ☐ Single click on a tool to select it for a single use, after which the pointer will be the selected tool.
- ☐ Double click on a tool for multiple uses, until another tool is selected from the tool box.

When moved over the image, the mouse cursor is accompanied with a symbol to indicate the function of the tool. For example, when you select a rectangle, a small square will appear next to the pointer.

Use the left mouse button to draw on the image.

The last region drawn will be the selected region and will be listed in the Region drop-down menu in the template bar. You can edit or delete the selected region by choosing **Edit** or **Delete** respectively from the **Analysis** menu.

Rectangle Drawing Tool

To draw a rectangular region:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to anchor one corner of the rectangle.
- ☐ Drag out the opposite corner of the rectangle with the mouse and release the mouse button.

Ellipse Drawing Tool

To draw an elliptical region:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to anchor one corner of the bounding rectangle of the ellipse.
- ☐ Drag out the ellipse and release the mouse button.

Irregular Drawing Tool

To draw an irregular region:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to begin drawing.
- ☐ Drag the mouse to enclose the ROI with an arbitrary shape.
- ☐ Draw the line to the starting point to close the irregular region.

If the border of the region crosses itself, the drawing process will stop, and the enclosed area will be the irregular region.

- ☐ Release and click the left mouse button to make a straight edge.
- ☐ Double click the left mouse button to close the irregular line with a straight line to the starting point.

ROI Drawing Tool

This tool will define a Region of Interest. A ROI defines the area in which the find process will automatically create regions. If no ROI is defined, the find process will look at the entire image. A ROI also defines the area used as the total area by “% of Total (ROI)” report option.

To create a ROI:

- ☐ Select  from the tool box.
- ☐ Press the left mouse button to anchor one corner of the rectangle.
- ☐ Drag out the other corner of the rectangle with the mouse and release the mouse button.

This will define a ROI. See the section *Finding Regions Automatically*, below for more information.

Qualifier Buttons

Below the region drawing tool icons in the tool box are qualifier buttons. These buttons determine whether the regions will be considered as unknowns (Unkwn) or as background (Bkgnd) regions.

Select “Bknd” in the tool box to create a background region. (You can change this selection after the region has been drawn. See the section *Editing Regions* (page R228).

More than one region can be identified as a background region. The total counts in the background regions will be divided by the total area of the regions to determine the background.

Labeling Regions

You can assign labels to individual regions within a group. You can also choose to print the labels on the image and in reports.

A label for a selected region can be added or edited in either the template bar or in the **Edit Region** window (Figure 6-3). Enter labels as you draw the regions or later by editing existing regions.

To enter labels as you draw regions:

- ☐ Draw a region, which will become the selected region. Then select the Label box in the template bar and enter a label into the box.
- ☐ Draw a second region and enter a label for that region from the keyboard. (The Label box will have the focus from the first time you clicked on it, and the text you enter will appear in the box.)

To edit labels for an existing group of regions without using the mouse for each region:

- ☐ Select a region by clicking on the region tag with the mouse. Selecting a region this way enables the function keys.
- ☐ Press **F6** or press **<Alt>-A,E** on the keyboard to open the **Edit Region** window. Enter a label for the selected region, and press **<Enter>**.
- ☐ Press **F9** to select the next region (or press **<Shift>-F9** to select the previous region). Enter a label.

Editing Regions

The **Edit Region** window (Figure 6-3) displays information about the selected region and allows you to change its properties.

To display the **Edit Region** window:

- ☐ Double click on the region's tag (when the pointer becomes a pointing finger).

Or,

- ☐ Single click on the region's tag.
- ☐ Select **Edit** from the template bar or **Edit Template** the **Analysis** menu.

The following information is displayed at the top of the window:

- The region's number and shape.
- The gross counts in the region.
- The area of the region in square millimeters.
- The coordinates of the region's center of gravity (centroid).

Any changes you make affect the parameters of the selected region only.

The selections at the **Edit Region** window are described below.

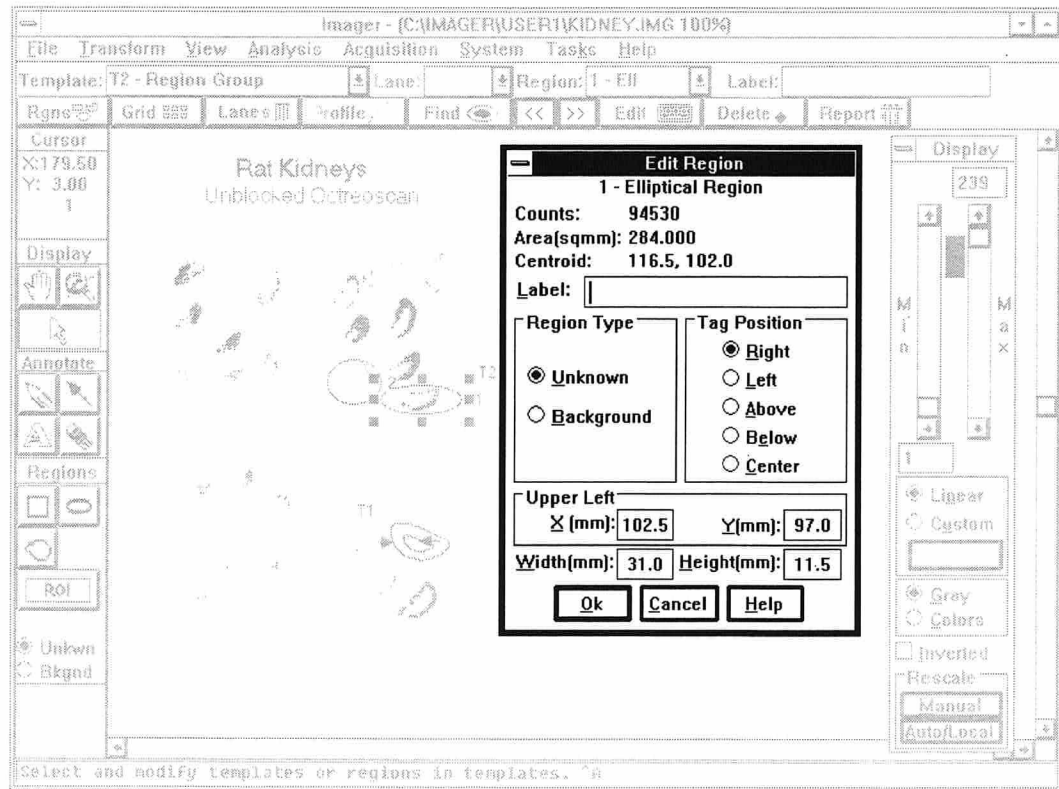


Figure 6-3. Edit Region Window.

SELECTION**DESCRIPTION OF USE****Label**

Edit the label assigned to the current region.

**Region Type:
Unknown/Background**

Select the region type: Unknown or Background. Regions are created as unknown or background depending on the region qualifier selected in the tool box when the region is drawn.

**Tag Position:
Right, Left, Above, Below,
Center**

Select the placement of the tag relative to the position of the region: to the Right, Left, Above, Below or in the Center.

**Upper Left: X(mm)
Y(mm)**

Edit the X and Y coordinates of the upper left corner of an ellipse or rectangle, or of the origin of an irregular region.

Width (mm)

Edit the width of a rectangle or ellipse. This box is blank when the region is irregular.

The width of the region is measured in millimeters.

Height (mm)

Edit the height of a rectangle or ellipse. This box is blank when the region is irregular.

The height of the region is measured in millimeters.

OK

Saves the parameters and updates the region. The region becomes the selected object on the image.

CANCEL

Aborts the operation. Closes the window.

Editing Regions Graphically

You can edit a region or group of regions graphically on the screen. You must use the pointer to edit a region graphically:

- ☐ Select  in the tool box.

To select a region group:

- ☐ Select the region group tag (T1, T2, etc.) with the pointing finger.

To select a region:

- ☐ Select the region tag with the pointing finger. Resizing handles are displayed around the region.

Moving and Copying Groups of Regions

If an entire group of regions is selected, the pointer will become a move/copy cursor when it is moved over the regions.

The move/copy cursor looks like this: 

The group of regions can be dragged using the left mouse button.

If you press the <Ctrl> key while dragging with the left mouse button (or if you only use the right mouse button), the entire group of regions will be copied to wherever you drag the mouse.

You can also move and copy individual regions.

Resizing Regions

The pointer will become a resizing cursor over the square resizing handles on rectangles or ellipses. You can then drag the edges or corners of the regions with the left mouse button to change the size of the region.

The resizing cursor looks like this: 

Finding Regions Automatically

Most samples, including two dimensional TLC plates, two dimensional electrophoresis gels, and tissue sections can be quantitated by placing regions on the image. You can quantitate regions by either 1) manually drawing regions within a group on the image, or 2) using the **Find Regions** window to automatically place regions on the image within a group.

The **Find Regions** window enables you to run the find regions process. This process enables the system to automatically find and create regions on an image.

The system uses three user-specified parameters as it automatically locates and creates regions of interest:

- Separation.
- Sensitivity.
- Background.

Separation ensures that all relevant pixels (those that contain the most significant number of counts) will be included in a region.

Sensitivity determines how well the system detects all regions of interest in an image. Higher degrees of sensitivity helps to insure that more regions will be found by the system.

Background determines the amount of background that will be allowed in the image. The amount of background you specify will be subtracted from every pixel in the region prior to processing.

You can specify the shape or type of region you want created:

- Irregular: Creates an irregularly shaped region that covers only those pixels included in the region.
- Elliptical: Creates the smallest possible elliptically shaped region that covers all of the pixels in the region.

The process of finding regions is described below.

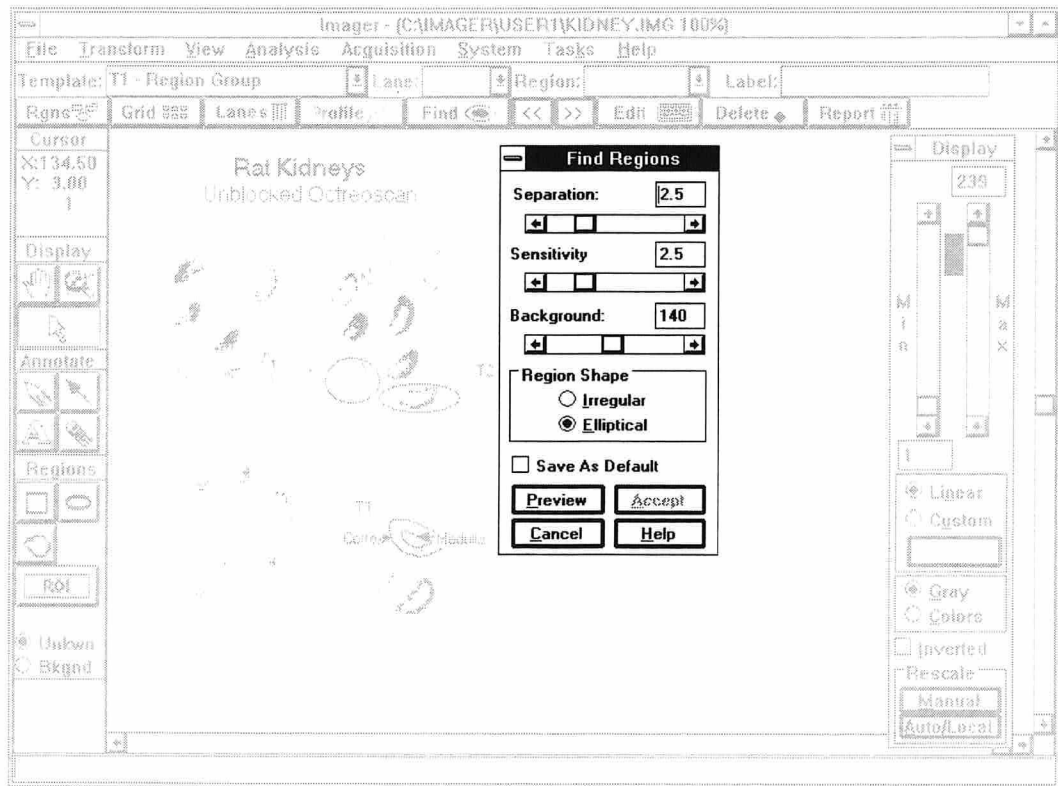


Figure 6–4. Find Regions Window.

How to Find Regions Automatically

The process of finding regions includes the following steps:

1. Create a region group.
2. Specify the parameters.
3. Preview the regions.
4. Accept the regions.

Each step is discussed below.

Create Region Group

To start the find regions process, you must first create a region group:

- ☐ Select the **Rgns** button from the template bar. The **Edit Group of Regions** window is displayed.
- ☐ Select **OK**.

A group tag is displayed over the image ("T1", for example). This tag represents the first region group. The regions that are created will be automatically anchored to this region tag.

Specify the Parameters

You must specify several parameters at the **Find Regions** window (Figure 6-4). To display this window:

- ☐ Select **FIND** from the template bar.

Specify the following parameters:

- ☐ At "Separation", click on the right arrow or the left arrow to increase or decrease the amount of separation, or use the slide control. You can also enter the value in the entry box.
- ☐ At "Sensitivity", click on the right arrow or the left arrow to increase or decrease the amount of sensitivity, or use the slide control. You can also enter the value in the entry box.
- ☐ At "Background", click on the right arrow or the left arrow to increase or decrease the level of background, or use the slide control. You can also enter the value in the entry box.

To specify the shape of the regions:

- ☐ Select "Irregular" (Region Shape) for irregularly drawn regions.
- ☐ Select "Elliptical" (Region Shape) for elliptically drawn regions.

To specify the current parameters as the default:

- ☐ Select "Save As Default". If this checkbox is selected when you close the **Find Regions** window (by selecting **ACCEPT**) the current parameter settings will be saved as your default settings.

 **Note:** *You can use the entire screen as your region of interest, or you can optionally select and draw a rectangle (ROI) over a portion of the image (see *Creating a Region of Interest*, below).*

Preview the Regions

Select **PREVIEW** to start the find regions process.

Selecting **PREVIEW** deletes any currently previewed regions. It also creates regions of interest based on the current parameters. Process bars will appear on the screen informing you of the % complete of the find regions process. You can terminate the process by selecting **CANCEL** in any of the process bars.

The regions will be displayed on the image so that you can see the results. These regions will not be a permanent part of the template until you select **ACCEPT**.

Accept the Regions

Select **ACCEPT** to save the current set of regions.

This selection is grayed (inactive) until a set of regions has been previewed. When you choose **ACCEPT**, the currently previewed regions will become a permanent part of the template and the **Find Regions** window will close.

If any regions already exist for this group of regions, a window will appear asking if the previously existing regions in the selected ROI should be deleted. Select **YES** to delete all regions that exist within the ROI. Select **NO** to retain all regions that exist within the ROI. Select **CANCEL** to terminate the process and return to the **Find Regions** window.

To reject the current set of regions:

- ☐ Select **CANCEL**. You can initiate the find regions process again.

Creating a Region of Interest

When you draw a specific ROI over an image, the find regions process will create regions within the specified area only. If no ROI is drawn, the find regions process will look at and use the entire image.

To draw a specific Region of Interest:

- ☐ Select  from the tool box.
- ☐ Point to an area of the image you are most interested in.
- ☐ Press the left mouse button to anchor one corner of the rectangle.
- ☐ Drag out the opposite corner of the rectangle with the mouse over the selected portion of the image and release the mouse button.

The rectangle (ROI) is drawn over the image.

Reporting Region Groups

See the Chapter *Reporting Results* for more information on reporting quantitative results obtained from regions.

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